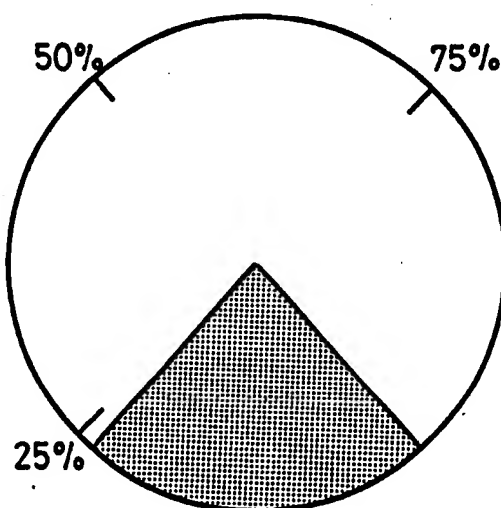




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : G01K 3/04, C12Q 1/42	A1	(11) International Publication Number: WO 92/05415 (43) International Publication Date: 2 April 1992 (02.04.92)
(21) International Application Number: PCT/GB91/01538 (22) International Filing Date: 10 September 1991 (10.09.91) (30) Priority data: 9020364.7 18 September 1990 (18.09.90) GB (71) Applicant (for all designated States except US): CAM- BRIDGE CONSULTANTS LIMITED [GB/GB]; Science Park, Milton Road, Cambridge CB4 4DW (GB). (72) Inventor; and (75) Inventor/Applicant (for US only) : LIVESLEY, David, John [GB/GB]; 17 Station Road, Swavesey, Cambridge CB7 5QT (GB). (74) Agent: KEITH W. NASH & CO.; Pearl Assurance House, 90-92 Regent Street, Cambridge CB2 1DP (GB).		(81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GB, GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), NO, SE (European patent), US. Published <i>With international search report.</i>

(54) Title: TIME TEMPERATURE INDICATION**(57) Abstract**

A time temperature indicator, for example for indicating product life, in which a label, embodied directly in or applied to a package, carries a substrate which is catalysed by an enzyme laid down in a progressive concentration to produce directly a differently coloured reaction product, the development of which progresses along or around the label in accordance with time and temperature conditions.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MG	Madagascar
AU	Australia	FI	Finland	ML	Mali
BB	Barbados	FR	France	MN	Mongolia
BE	Belgium	GA	Gabon	MR	Mauritania
BF	Burkina Faso	GB	United Kingdom	MW	Malawi
BG	Bulgaria	GN	Guinea	NL	Netherlands
BJ	Benin	GR	Greece	NO	Norway
BR	Brazil	HU	Hungary	PL	Poland
CA	Canada	IT	Italy	RO	Romania
CF	Central African Republic	JP	Japan	SD	Sudan
CG	Congo	KP	Democratic People's Republic of Korea	SE	Sweden
CH	Switzerland	KR	Republic of Korea	SN	Senegal
CI	Côte d'Ivoire	LI	Liechtenstein	SU ⁺	Soviet Union
CM	Cameroon	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
DE*	Germany	MC	Monaco	US	United States of America
DK	Denmark				

⁺ Any designation of "SU" has effect in the Russian Federation. It is not yet known whether any such designation has effect in other States of the former Soviet Union.

- 1 -

Title Time Temperature IndicationField of the invention

This invention concerns time temperature indication and relates to a time temperature indicator, more especially for chilled or frozen foods. The invention also relates to a label incorporating such an indicator, and a method of time temperature indication.

Background to the invention

A time temperature indicator is a device for indicating temperature conditions with respect to time. In the case of chilled or frozen food products, for example, it is important to be able to establish if a product has been subject to proper temperature conditions over a period of time, since this determines the shelf life of the product.

One time temperature indicator is known from U.K. Specification No. 1366797. The principle of this indicator is an enzyme catalysed reaction. Enzyme catalysed reaction rates are affected by a number of parameters, but given a standard chemical environment, the major parameter is temperature. Enzymes have an optimum temperature range at which they operate, and below that range reaction rates are considerably slowed. The relationship between temperature and reaction rate may be plotted and enzymes selected for which the relationship of

- 2 -

reaction rate to temperature over the temperature range of 4-20°C is linear. This is the expected temperature range for chilled food products. If however an enzyme system were selected where the relationship between temperature and reaction rate is non-linear, it is possible to make allowances for this within the system.

The disadvantage of the indicating system known from the above specification is that the enzyme catalysed reaction produces a change in pH which then requires a second reaction to indicate the pH change which has occurred.

The invention

According to the invention, a time temperature indicator is provided which is based on by an enzyme catalysed reaction in which the enzyme catalyses reaction of a substrate to produce directly a differently coloured reaction product.

Thus, in one aspect the invention provides a time temperature indicator, characterised by a substrate and an enzyme which catalyses reaction of the substrate to produce directly a reaction product of different colour to the substrate.

The enzyme catalysed reaction is chosen such that it fulfils one of the following conditions:-

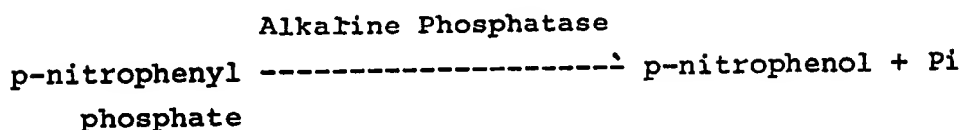
1. The substrate is either colourless or possesses a pale colour, whilst the product of the reaction is strongly coloured.
2. The substrate is strongly coloured, and the reaction

- 3 -

product is pale or colourless.

3. The substrate and reaction product both have strong colours, but the colours are distinctly different.

An example of such a reaction is:



p-nitrophenol is normally colourless, but has a strong yellow colour under alkaline conditions.

The enzyme is conveniently provided in association with the substrate at a range of concentrations, such that the rate of colour change varies only with time given a constant temperature.

The principal advantage of the invention over the known indicator system, which uses lipase to degrade a lipid and then measures the resulting pH change, is that it gives a direct reading, not affected by the variability and relative insensitivity of pH measurement.

Another advantage inherent in the invention is that, provided a suitable enzyme and substrate system is selected, it is possible to have a fully food compatible system such that all reagents are natural food products and no food contamination problems exist.

Still another advantage is that, given a suitable carrier system for the enzyme and substrate systems, they may be directly incorporated into a package and are therefore not

- 4 -

removable for tampering purposes. They may, however, be produced in an adhesive label format if required.

The use of non-impact printing technology, specifically ink jet printing, to lay down the enzyme component in a label makes the system inherently flexible, and it is possible to vary labels at will such that there is no machine downtime if the storage conditions or shelf life of the product need to be altered. It is considered possible to produce devices with life times extending to several weeks or months.

The label may have any format that is required, due to the flexibility of the production process. Thus, the following physical formats of the label may be produced:-

1. A self adhesive paper label, where the paper is impregnated with the enzyme substrate, and then the enzyme solution applied to the label using one of a variety of printing techniques to give a single layer label with the required graphics. The finished labels may then be stored frozen until applied to a food package.
2. A self adhesive label impregnated with the enzyme substrate, and a clear self adhesive laminate label printed with the required enzyme pattern, each component being stored separately in a cool dry place until applied to the package. Lamination of the two label layers activates the enzyme reaction.
3. A paper label containing the enzyme substrate may be laminated on to the product package, during the production process, and then overprinted with the enzyme solution prior to the package entering a chill tunnel.

- 5 -

4. The enzyme substrate may be directly incorporated into an ink formulation, such that it constitutes a label directly printed on to the packaging material. The enzyme solution may then be overprinted on to the substrate patch at a convenient later time, using a second ink formulation. This format has the specific advantage that it is integral with the packaging material, and so cannot be removed from the food package, possibly to be tampered with. It is desirable to ensure that the labelling sets in a permeable and water proof fashion on curing.

The graphics format of the label is also extremely adaptable because of the use of ink jet printing. Preferred formats are:

1. A blush label. Using grey scale printing technology and a carefully selected substrate which allows limited wicking, it is possible to produce a label such that colour change runs up the label with time. The colour therefore appears to "blush" up the label. Thus, the distance the colour front has advanced up the label can be used to quantify how much of the total time/temperature history has been used.

Another advantage of this format is that it can be utilised to run into the bar code on the package, thus interfering with scanning equipment, so that out of date articles will not pass through a check out scanner.

2. A clock format. This is a circular format where colour change develops like the sweeping action of a clock hand. This format can if desired be quantified to show

- 6 -

the percentage of shelf life remaining.

However, many other formats are also possible, including that of a simple message which becomes visible at the end of shelf life, or a window which becomes coloured at the end of shelf life.

In a further aspect the invention provides a method of time temperature indication, characterised by use of an enzyme substrate to produce directly a reaction product of different colour to the substrate, and monitoring change of colour of the substrate with time.

Some specific examples of label format are shown by way of illustration in Figures 1 to 6 of the accompanying drawings, in which each Figure shows one possible format.

The illustrated embodiments each comprise a label impregnated with p-nitrophenol and carrying a layer of alkaline phosphatase laid down, eg by ink jet printing, in a pattern of varying concentration.

Figures 1 to 3 show three examples of blush label, with enzyme concentration increasingly progressively from left to right. In the label of Figure 1, colouring progresses from left to right to indicate the days of shelf life remaining, assuming the product is stored at 4°C. The label of Figure 2 is used to indicate acceptance criteria for the product, at the stages of manufacture, distribution and offer for sale. Figure 3 shows a label marked to show the percentage of remaining shelf life, again as colouring progresses from left to right in accordance with time/temperature conditions of storage.

- 7 -

In the label of Figure 4, a printed message appears at the end of shelf life, whilst in the label of Figure 5 a coloured window appears at the end of shelf-life.

Figure 6 shows a label having a clock format, wherein enzyme concentration increases progressively around the clock and colouring progresses around the clock to indicate usable product life as a percentage of its full life. Colouring may commence, for example, approximately in the five o'clock position, for example, so that in the illustration, when this colouring has extended clockwise to beyond the seven o'clock position, nearly 25% of the product life has been used up, depending on the time for which the product has been stored since manufacture and the temperature conditions which have prevailed during that time period.

- 8 -

Claims

1. A time temperature indicator in which an enzyme catalysed reaction is employed, characterised by a substrate and an enzyme which catalyses reaction of the substrate to produce directly a reaction product at different colour to the substrate.
2. An indicator according to claim 1, characterised in that the substrate is pale or colourless and the reaction product is strongly coloured.
3. An indicator according to claim 2, characterised in that the substrate is p-nitrophenyl and the enzyme is alkaline phosphatase.
4. An indicator according to any one of claims 1 to 3, characterised in that the enzyme is provided in association with the substrate in a range of concentrations, such that the rate of colour change varies only with time given a constant temperature.
5. An indicator according to any one of claims 1 to 4, in combination with a carrier for the substrate and enzyme.
6. An indicator according to claim 5, characterised in that the carrier is incorporated directly into a package for which time temperature indication is required.
7. An indicator according to claim 5, characterised in that the carrier comprises an adhesive label.

- 9 -

8. An indicator according to any of claims 1 to 7, characterised in that the enzyme is laid down by ink jet printing.
9. An indicator according to any of claims 1 to 8, characterised in that the indicator is embodied in a format in which colour change progresses along or around the format in accordance with prevailing time temperature conditions.
10. A method of time temperature indication, characterised by use of an enzyme catalysed reaction in which the enzyme catalyses reaction of a substrate to produce directly a reaction product of different colour to the substrate, and monitoring change of colour of the substrate with time.

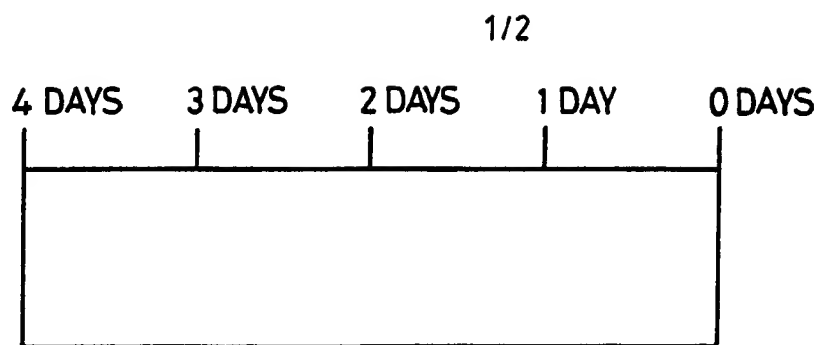


Fig. 1

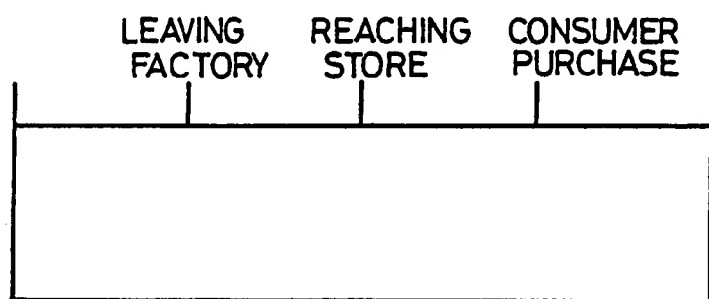


Fig. 2

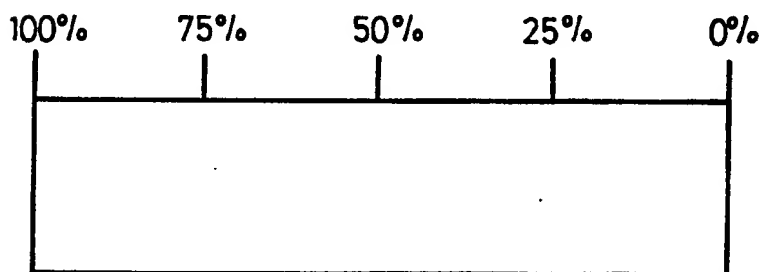


Fig. 3



Fig. 4

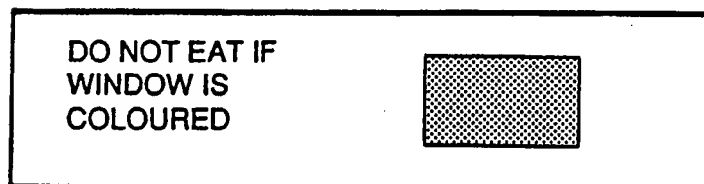


Fig. 5

2/2

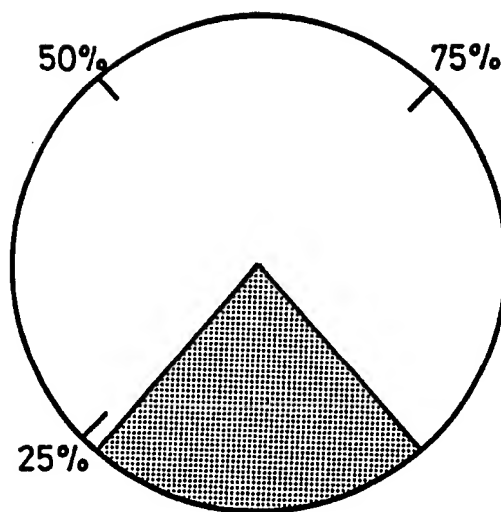


Fig. 6

INTERNATIONAL SEARCH REPORT

PCT/GB 91/01538

International Application No

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int.Cl. 5 G01K3/04; C12Q1/42		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl. 5	G01K ; C12Q ; G04F	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
A	US,A,4 826 762 (A.M. KLIBANOV) 2 May 1989 see the whole document ---	1,2,4-7
A	EP,A,0 132 830 (LA ROSSA, DENISE DE GUIRE) 13 February 1985 see page 3, line 8 - page 4, line 36 see page 5, line 34 - page 7, line 19 see page 9, line 28 - page 10, line 34 ---	1-4
A	EP,A,0 310 014 (BEHRINGWERKE) 5 April 1989 Introduction; figure 1 ---	1-4
<p>¹⁰ Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
12 DECEMBER 1991	17. 01. 92	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	PANDOLFI	

Form PCT/ISA/210 (second sheet) (January 1985)

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

GB 9101538
SA 51134

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 12/12/91

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-4826762	02-05-89	None	
EP-A-0132830	13-02-85	US-A- 4555484	26-11-85
		CA-A- 1218290	24-02-87
		DE-A- 3467773	07-01-88
		JP-A- 60043400	07-03-85
EP-A-0310014	05-04-89	DE-A- 3732871	20-04-89
		AU-A- 2295888	06-04-89
		JP-A- 1115962	09-05-89

EPO FORM P0479

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82